DEVELOPMENT AND EVALUATION OF ATAZANAVIR LOADED SOLID LIPID NANOPARTICLES

MEDIPALLI VISWAJA*[1], D.V.R.N. BHIKSHAPATHI[1], ARJUN GOJE[1], RANGU NIRMALA[2]

[1]TRR College of Pharmacy, Meerpet, Hyderabad, Telangana-500097, India.
[2]Surabhi Dayakar Rao College of Pharmacy, Gajwel- 502312, Siddipet, Telangana. India

*Corresponding author: M. Viswaja, Department of Pharmaceutics, TRR College of Pharmacy, Meerpet, Hyderabad, Telangana, Email: vishureddy24@gmail.com

ABSTRACT

The primary motive is to develop the solid lipid nanoparticles formulation of atazanavir with enhanced dissolution rate. The prepared Atazanavir SLNs formulation batches (*i.e.,*18 formulations), composed of solid-lipid (Tri-stearin/ Witepsol E85), surfactants and stabilizer (Cremophor RH40, Pluronic F68, Tego care 450 and Tween 80) were fabricated employing hot homogenization followed by the ultrasonication. All the formulations were evaluated for particle Size, zeta potential, drug content, Entrapment Efficiency and in-vitro drug release. Optimised formulation was subjected for particle Size, zeta potential, FTIR, SEM and stability studies. The mean particle size, zeta potential, drug content, entrapment efficiency (EE), and in-vitro drug release profile of atazanavir loaded SLNs were found to be ranged from 59.42 nm – 146.29 nm., -15.1mV to -23.57mV, 94.61 to 99.72%, 94.83 to 99.52%, .and 54.90 to 81.40% at the end of 12 hours respectively. The amount of drug released from the SLN dispersions generated using Witepsol E85 as the lipid matrix, with tego care 450 as the surfactant (as shown highest for F18= 99.72±0.46%), was significantly controlled and regulated than that from the atazanavir-pure drug solution (31.09±1.16%). The optimized formulation (F18) exhibited particle size of 79.21 nm, zeta potential -16.1mV and FTIR studies revealed that there is no incompatibility between drug and excipients, SEM images exhibited nanoparticles to be more porous and in spherical shape. Stability studies indicated F18 formulation was stable for 3months.The results implied that the proposed way of SLN preparation could be considered as a proper method for production of atazanavir loaded colloidal carrier system.

Key words: Atazanavir, solid-lipid nanoparticle, Particle size, In-vitro drug release, FTIR studies

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INTRODUCTION

Aqueous solubility and first-pass metabolism are the major factors responsible for poor oral bioavailability of lipophilic drugs. Lipid-based formulations showed success in reducing the inherent limitations of slow and incomplete dissolution of poorly water-soluble drugs by facilitating the formation of solubilized phase and further, improving the absorption [Charman and Porter, 1996].

Solid lipid nanoparticles (SLNs) are emerging as alternative carriers to other colloidal drug systems, in controlled and targeted drug delivery. These are in submicron size range (50- 1000nm) and are made of biocompatible and biodegradable materials capable of incorporating lipophilic and hydrophilic drugs. SLNs combine the advantage of different colloidal carriers, vesicular carriers and polymeric carriers [Muhlen et al., 1994] as physiologically acceptable systems and also in imparting the controlled release of drug from lipid matrix [Müller et al., 2000]. Furthermore, these systems enhance the lymphatic transport of the lipophilic drugs, irrespective of the route of administration, and therefore increases the systemic availability of drug molecule.

The model drug for the current study had been selected from the biopharmaceutical classification system class II. Atazanavir sulphate is a novel azapeptide HIV protease inhibitor with high specificity which prevents multiplication of HIV. Atazanavir sulphate is an antiretroviral drug

which belongs to class-2 drugs, associated with poor solubility. Atazanavir sulphate has less bioavailability due to pH-dependent solubility and absorption [raja et al., 2003]. To overcome hepatic first-pass metabolism and to enhance oral bioavailability, lipid–based drug delivery systems like solid lipid nanoparticles can be used. These systems enhance the lymphatic transport of the lipophilic drugs and therefore increase the bioavailability [Üner and Yamen, 2007]. The prime objective of the present study is to develop suitable solid-lipid nanoparticle (SLN) formulation for Azatanavir for enhanced drug dissolution rate.

MATERIALS & METHODS

Atazanavir was gift sample from Hetero Labs Ltd, Hyderabad. Witepsol E85, Tri-stearin Cremophor RH 40, Pluronic F68, Tego care 450, tween 80 were purchased from Gattefosse, Mumbai. All the reagents used were of analytical grade

Preparation of atazanavir loaded solid lipid nanoparticles

Atazanavir loaded SLN were prepared by hot homogenization followed by the ultrasonication method. Atazanavir and solid lipid (Tri-stearin/ Witepsol E85) were dissolved in a mixture of methanol and chloroform (1: 1). Organic solvents were completely removed using a rotary flash evaporator. The embedded lipid layer was melted by heating to 5°C above the melting point of the lipid. An aqueous phase was prepared by dissolving the surfactants and stabilizer (Cremophor RH40, Pluronic F68, Tego care 450 and Tween 80 respectively) in distilled water (sufficient to produce 30 ml) and heating to the same temperature of the oil phase. The hot aqueous phase was added to the oil phase and homogenization was performed (at 2500 rpm and 70°C) using a mechanical stirrer for 30 minutes. The coarse oil in water emulsion so obtained was sonicated using probe sonicator for 25 minutes. Atazanavir loaded SLN was finally obtained by allowing the hot nanoemulsion to cool to room temperature, and was stored at 4°C in the refrigerator. The composition of the different formulation has been given in Table 1.

In all SLN formulations the lipid concentration was kept constant (as 1.5% w/v) [Muller et al 2000]

Evaluation of atazanavir-loaded solid lipid nanoparticles

Measurement of particle Size, Polydispersity Index and zeta potential

The particle size and polydispersity index (PDI) of the SLN were determined by photon correlation spectroscopy (PCS) with a Zetasizer Nano ZS-90 (Malvern Instruments Ltd., Worcestershire, UK). Prior to analysis, samples of all SLN formulations were diluted with double distilled water. The zeta potential measurements were done by laser-doppler-anemometer coupled with Zetasizer Nano ZS-90 (Malvern Instruments Ltd., Worcestershire, UK) to validate the electrophoretic mobility of particles. All the analysis were repeated in triplicate. [Daswadkar et al 2020], [Schubert et al2003]

Drug Content

1 ml SLNs dispersion was taken into 100 ml volumetric flask and volume was made up with methanol. It was sonicated for 5 min in bath sonicator. Solution was filtered through cellulose whatman filter paper (0.45µ) and filtrate was analyzed at UV-visible spectrophotometer at 298 nm [Joseph et al 2017]

Entrapment Efficiency (%)

The entrapment efficiency of SLN dispersion was determined by the centrifugation method [Vivek et al 2007] [Yang et al 2010]. SLN dispersion (containing an equivalent to 5 mg of drug) was centrifuged at 20000 rpm for one hour in a refrigerated centrifuge to collect the supernatant liquid. The collected liquid was filtered to measure the free drug concentration after suitable dilution with a fresh phosphate buffer saline pH 6.8. The absorbance was measured at 259 nm in a UV spectrophotometer to calculate the entrapment efficiency using the following formula:

Entrapment efficiency = Wt. of drug incorporated/ Wt. of drug initially taken × 100

In vitro drug release

The invitro-drug release of atazanavir from different SLN dispersions was determined using the dialysis bag diffusion technique. An accurately weighed amount of Atazanavir-loaded SLN dispersions containing the drug equivalent to

300mg was transferred to a dialysis bag and sealed. The sealed bag was then suspended in a beaker containing 250 ml of phosphate buffer saline pH 6.8 and stirred at a constant speed of 50 rpm at 37°C ± 0.5°C. Aliquots were withdrawn at predetermined intervals from the receptor compartment up to 12 hours and the same was replaced with fresh buffer. Then the drug content was determined spectrophotometrically by measuring the absorbance at 298 nm using the respective receptor medium as a blank, to calculate the amount of drug released from the nanoparticles [Jain et al 2018]

Characterization of optimized atazanavir SLN formulation

Emulsion droplet size analysis/particle size determination

The selected optimised formulation that indicated self-emulsification and non-precipitation was subject to droplet size analysis. The formulation, 100 µL was diluted to 250 mL in a beaker and gently mixed using a glass rod. The resultant emulsion was then subjected to particle size analysis using Malvern zetasizer (Malvern, UK). All studies were repeated for six times, with good agreement being found between measurements [Jeevana JB et al, 2011].

Zeta potential determination

The zeta potential ζ of the diluted SLN formulations was measured using a Malvern zetasizer (Malvern, UK). The SLN were diluted with a ratio of 1:2500 (v/v) with distilled water and mixed for 1 min using a magnetic stirrer. Zeta potential of each SLN was determined in triplicate [Jeevana JB et al, 2011].

Drug compatibility by FTIR

The FTIR of pure drug and optimised formulation scanned and compared to check any incompatibility between the drug compound and excipients used [SellappanV et al, 2014].

SEM studies

The SEM analysis of prepared SLN was performed for morphological studies. The formulations are poured in to circular aluminum stubs using double adhesive tape, and coated with gold in HUS -5 GB vacuum evaporator, and observed in Hitachi S-3000 N SEM at an acceleration voltage of 10 Kv and a magnification of 5000X [Nair, R et al 2012]

Accelerated Stability Studies

As per ICH guidelines (2003), the samples for stability analysis must be exposed to an environment of accelerated stability studies: -40 °C±2 °C/75%RH±5%RH for a period of 3months. As per the standard protocol, the samples must be analysed at 0, 1, 2 and 3months' time points. Accelerated stability studies were performed for the final optimized formulation.

RESULTS AND DISCUSSION

Particle size analysis

The particle size ranged from 59.42 nm – 146.29 nm. There was a significant difference in the size of the particles, with change in the lipids and surfactant concentration.

Effect of lipids on the particle size of atazanavir SLN

Atazanavir-loaded solid lipid nanoparticles, prepared by using tri-stearin as the lipid matrix, resulted in larger particle size compared to SLN prepared by using witepsol as the lipid matrix, with all type of surfactants studied. The particle size of various SLNs prepared with different lipids was in the order of tri-stearin> witepsol E85.

Effect of surfactants on the particle size of atazanavir SLN

SLN dispersions with increase in surfactant concentration increased in particle size and SLN's prepared using tego care 450 as surfactant with witepsol lipid [F16 to F18] showed lower particle size than the other surfactants.

Zeta potential

Zeta potential is a key factor to evaluate the stability of colloidal dispersion. It was currently admitted that zeta potentials between -15.1mV to -23.57mV were required for full electrostatic stabilization. However, many experiments demonstrated that not only electrostatic repulsion dominated the stability of nanoparticles, the use of steric stabilizer also favored the formation of stable nanoparticle dispersion.

In these studies, it seemed that the value of zeta potential of atazanavir loaded SLNs was not sufficient to keep the particles dispersing stably.

However, the particle size did not change significantly within 30 days, which should contribute to the following point. High surfactant mixture can easily compensate for missing electrostatic repulsion to stabilize the dispersion for long time. Tego care 450 provides a steric stability for maintaining the stability of SLN.

Drug content

The drug content of all formulations ranged between 94.61 to 99.72% with maximum value exhibited by F18. It is observed that higher is the surfactant concentration more is the drug content

Entrapment efficiency

Entrapment efficiency is an important parameter for characterizing solid lipid nanoparticles. In order to attain optimal encapsulation efficiency, several factors were varied, including the type and concentration of the lipid and surfactant material used. The entrapment efficiency of all the prepared SLN formulations. The entrapment efficiency of the SLN dispersions was found to be in the range of 94.83 to 99.52%.

Effect of lipids and surfactants on drug content and entrapment efficiency

All SLN formulations prepared with a witepsol E85 showed better drug content and entrapment efficiency than formulation with tri-stearin. It is also observed that higher concentration of surfactant showed higher entrapment efficiency irrespective of the type of surfactant. This showed that the increase in the concentration of the surfactant increased the entrapment efficiency. This may be due to the increase in the solubility of the drug in the lipid on increasing the concentration of the surfactant. This result was in agreement with the results obtained by [Abdelbary et al 2009]. Formulations containing cremophor RH40 as surfactants showed lower entrapment efficiency compared to the other surfactants. Hence the entrapment efficiency of various SLNs stabilized with different surfactants, and decreased in the order of tego care 450> pluronic F68 > cremophor RH40.

In vitro drug release

The in vitro drug release profile of atazanavir from various SLN formulations is shown in (Figure 1). The in vitro release of atazanavir from the SLN dispersion was found to be in the range of 54.90 to 81.40% at the end of 12 hours.

Influence of surfactants on in vitro drug release

Formulations [F10-F18] prepared by using witepsol E85 as a lipid matrix, with cremophor RH40, pluronic F68 and tegocare 450 in higher amounts as stabilizers showed a higher drug release from Atazanavir-loaded SLN. This showed that the increase in the concentration of the surfactant there was an increase in the drug release from the SLN.

Influence of lipids on in vitro drug release

Formulations containing witepsol E85 as a lipid matrix exhibited higher drug release than the formulations containing tri-stearin as the lipid matrix. Witepsol E85 produced less ordered crystals than tri-stearin, leading to lower drug expulsion from the imperfect lattice, contributing to the prolonged release of the lipophilic drug. The order of percentage of drug release was Witepsol E85 > tri-stearin on the basis of the lipid matrix. Thus, it could be well concluded that the amount of drug released was much slower and controlled from the SLN dispersions prepared by using Witepsol E85 as the lipid matrix, with tego care 450 as the surfactant (as observed highest for F18= 99.72±0.46%), than that from the atazanavir-pure drug solution (31.09±1.16 %) [Figure 1]

Characterization of optimized formulation

Particle size and zeta potential of atazanavir optimized formulation

Globule size distribution and zeta potential of optimised F18 formulation was analysed and Figures 2,3 depict the optimized formulation's droplet size, and zeta potential respectively.

The mean globule size of F18 was 79.21 nm indicating nanoparticle range that facilitates absorption. The zeta potential (mean) values of SLN formulations were found to be in between -16.1 mV. The zeta potential value > 5 mV provide an excellent stability [Michalek M et al, 1996], [Chansiri G et al, 1999].

FTIR Spectroscopy

The FTIR spectrum of pure drug atazanavir showed several characteristic peaks as shown in Fig. 4 and it had characteristic peaks at 3423 cm^{-1} (-OH stretching), 3358 cm^{-1} (NH stretching),2964 cm^{-1} (C_N stretching),1712 cm^{-1}

(C=O stretching), peaks around 3800 cm^{-1} for aromatic C-H stretching,1620 cm^{-1} (CO-N stretching). The presence of prominent characteristic peaks confirming the purity of atazanavir as per the established standards.

The compatibility study carried out by comparing FTIR spectra of pure drug (figure 4) and optimised formulation (figure 5). The optimised formulation shows all principal peaks present in the FTIR of pure drug indicating the compatibility.

Scanning electron microscopy

The scanning electron microscopic pictures of atazanavir optimised SLN formulation F18 are presented in Figure 6A and 6B. SEM studies showed that the atazanavir-loaded solid lipid nanoparticles had a spherical shape with a smooth surface.

Accelerated Stability Studies

Stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and enables recommended storage conditions.

No visible physical changes were observed in all the formulations withdrawn from the humidity chambers. The samples were assayed for %entrapment efficiency, % drug content and in-vitro drug release and the results are shown in Table 2. No significant difference was observed after storage at accelerated conditions at 40±2° C/75±5% RH for a period of six months.

CONCLUSION

In this study, the atazanavir loaded SLNs were designed and prepared by the hot homogenization followed by ultrasonication technique. The amount of drug released from the SLN dispersions created using Witepsol E85 as the lipid matrix and Tego Care 450 as the surfactant was greatly controlled and regulated compared to that from the atazanavir-pure drug solution (31.09±1.16%), as evidenced in the maximum value for F18= 99.72±0.46%. The optimized formulation (F18) exhibited particle size of 79.21 nm, zeta potential -16.1mV. FTIR studies revealed that there is no incompatibility between drug and excipients, The Morphology of optimized SLNs was roughly spherical in shape. Stability studies indicated formulation was stable for 3months. Atazanavir's improved drug release from solid lipid nanoparticles demonstrated the possibility of lipid-based nanoparticles as a viable carrier in enhancing oral delivery of this poorly soluble medication.

REFERENCES

1. Charman, W.N., & Porter, C.J.H. (1996). Lipophilic prodrugs designed for intestinal lymphatic transport. Adv. Drug Deli. Rev., 19(2)
2. Mühlen, A.Z., Schwarz, C., & Mehnert, W. (1998). Solid lipid nanoparticles (SLN) for controlled release drug delivery - drug release and release mechanism. Eur. J. Pharm. Biopharm., 45:149-155.
3. Müller, R.H., Mäder, K., & Gohla, S. (2000). Solid lipid nanoparticles (SLN) for controlled drug delivery a review of the state of the art. Eur. J. Pharm. Biopharm., 50:161-177
4. Raja A, Lebbos J, Kirkpatrick P. Atazanavir sulphate nature reviews. News Anal 2003; 2:857-8.
5. Uner, M., & Yener, G. (2007). Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. Int. J. Nano., 2:289-300.
6. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery – A review of the state of the art. Eur J Pharm Biopharm 2000;50:161-77
7. Daswadkar Shubhangi C, Atole Abhijit Vasant, Formulation and Evaluation of Solid Lipid Nanoparticles of Olanzapine for the Treatment of Psychosis, Journal of Drug Delivery & Therapeutics. 2020; 10(5-s):25-31
8. Schubert M, Müller-Goymann C. Solvent injection as a new approach for manufacturing lipid nanoparticles-evaluation of the method a process parameter. Eur J Pharm Biopharm. 2003; 55(1):125–131.
9. Joseph E, Reddi S, Rinwa V, Balwani G, Saha R, Design and invivo evaluation of solid

lipid nanoparticulate systems of Olanzapine for acute phase schizophreniea treatment: Investigations on antipsychotic potential and adverse effects, European Journal of Pharmaceutical Sciences, 2017; 1(1):1-29.

10. Vivek K, Reddy H, Murthy RSR, "Investigations of the Effect of the Lipid Matrix on Drug Entrapment, In Vitro Release, and Physical Stability of Olanzapine-Loaded Solid Lipid Nanoparticles" AAPS PharmSciTech 2007; 8 (4):1-9.
11. Yang C, Zhao X, Hu H, et al. Preparation, Optimization and Characteristic of Huperzine A Loaded Nanostructured Lipid Carriers. Chem Pharm Bull. 2010; 58(5):656–661.
12. Jain, Hemant & Taware, Madhuri. (2018). Formulation Development And Evaluation Of Gastro-Retentive Dosage Form Of Atazanavir Sulphate. International Journal Of Pharmacy and Pharmaceutical Sciences. 10. 60. 10.22159/ijpps.2018v10i1.21179.
13. Jeevana JB, Sreelakshmi K. Design and evaluation of self-nanoemulsifying drug delivery system of flutamide. J Young Pharm. 2011;3(1):4-8.
14. SellappanV and Mohamed A: Development and evaluation of Ritonavir mucoadhesive microspheres. Asian Journal of Pharma Clinical Research 2014; 7(5): 47-52
15. Nair, R., Kumar, A.C., Priya, V.K. et al. Formulation and evaluation of chitosan solid lipid nanoparticles of carbamazepine. Lipids Health Dis **11,** 72 (2012).
16. ICH Harmonized Tripartite guideline on "Stability Testing of NewDrug Substances and Products Q1A (R2)", 6February**2003**
17. Abdelbary G, Fahmy RH. Diazepam – loaded solid lipid nanoparticles: Design and characterization. AAPS PharmSciTech 2009; 10:211-9.
18. Michalek M, Stachurski J. The effect of the zeta potential on the stability of nonpolar oil in water emulsion. J Colloid Interface Sci. 1996; 184:433–6.

Chansiri G, Lyons RT, Patel MV, Hem SL. Effect of surface charge on the stability of oil/water emulsions during steam sterilization. J Pharm Sci. 1999; 88:454–8.

Table 1: Composition of atazanavir solid lipid nanoparticles (F1-F18)

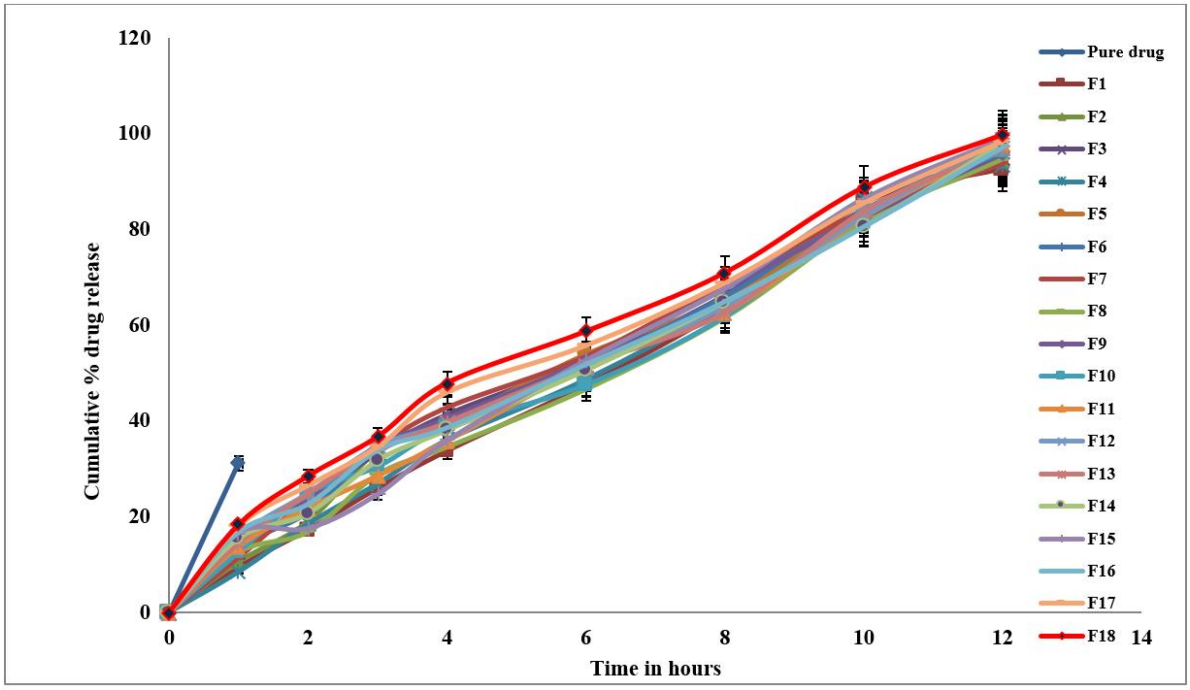
Formulation code	Doravirine (mg)	solid lipid Tri-stearin (% w/v)	solid lipid Witepsol E85 (% w/v)	Cremophor RH40	surfactant Pluronic® F-68 (% w/v)	Tegocare 450 (% w/v)	Tween 80 (ml)	Chloroform: Methanol (1:1)	Distilled Water (mL)
F1	300	1.5		2			0.5	20	Q. S
F2	300	1.5		4			0.5	20	Q. S
F3	300	1.5		6			0.5	20	Q. S
F4	300	1.5			2		0.5	20	Q. S
F5	300	1.5			4		0.5	20	Q. S
F6	300	1.5			6		0.5	20	Q. S
F7	300	1.5				0.5	0.5	20	Q. S
F8	300	1.5				1	0.5	20	Q. S
F9	300	1.5				1.5	0.5	20	Q. S

F10	300		1.5	2			0.5	20	Q. S
F11	300		1.5	4			0.5	20	Q. S
F12	300		1.5	6			0.5	20	Q. S
F13	300		1.5		2		0.5	20	Q. S
F14	300		1.5		4		0.5	20	Q. S
F15	300		1.5		6		0.5	20	Q. S
F16	300		1.5			0.5	0.5	20	Q. S
F17	300		1.5			1	0.5	20	Q. S
F18	300		1.5			1.5	0.5	20	Q. S

Table 2: Storage at 40±2° C/75±5% RH for 3 Months

Retest time for optimized formulation F18	% Drug content	%Entrapment efficiency	In-vitro drug release (%)
0 days	99.72±1.29	99.52±2.94	99.72±0.46
30 days	99.53±0.60	99.16±0.78	99.30±1.62
60 days	98.85±0.27	99.09±1.965	98.89±2.69
90 days	98.52±1.64	98.84±0.23	98.37±0.27

Above parameters are communicated as Average ± Standard Deviation; (n=3)



Above parameters are communicated as Average ± Standard Deviation; (n=3)

Figure 1: Cumulative percentage drug release of atazanavir pure drug and atazanavir SLN formulations (F1-F18)

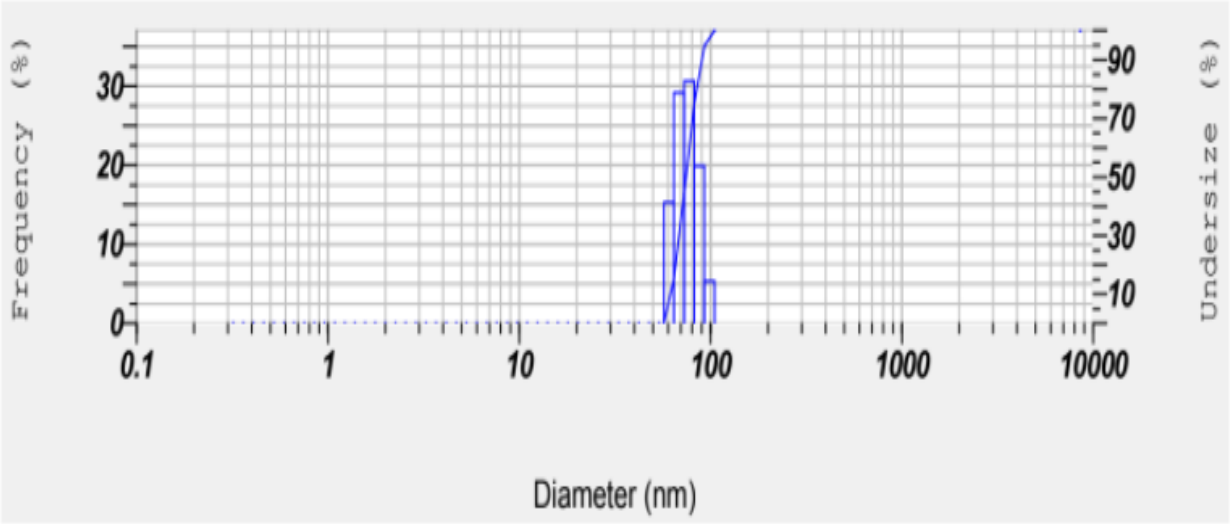


Figure 2: Particle size of optimised SLN formulation of atazanavir (F18)

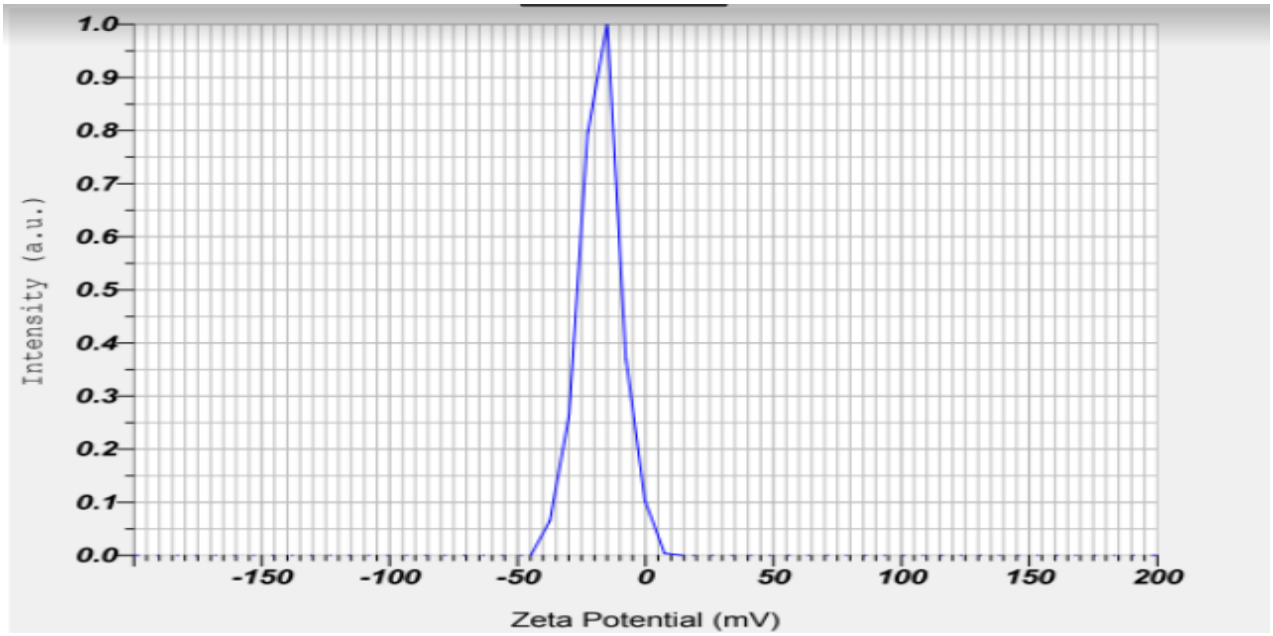


Figure 3: Zeta potential of optimised SNEDDS formulation of doravirine (F18)

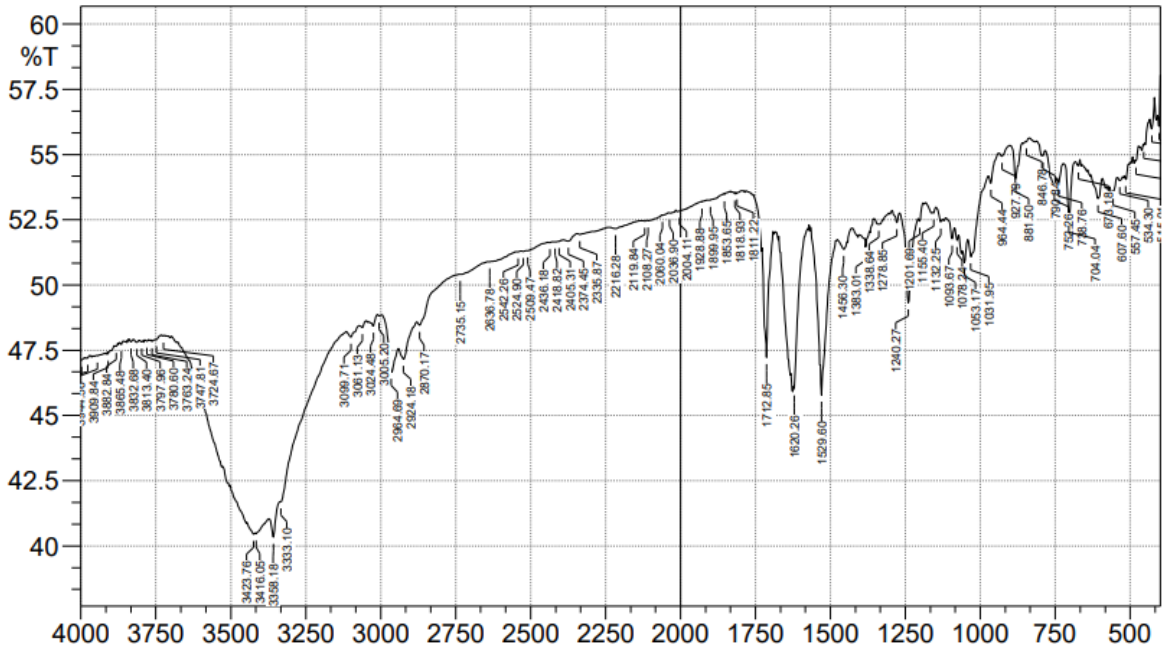


Fig 4: FTIR spectrum of pure drug atazanavir

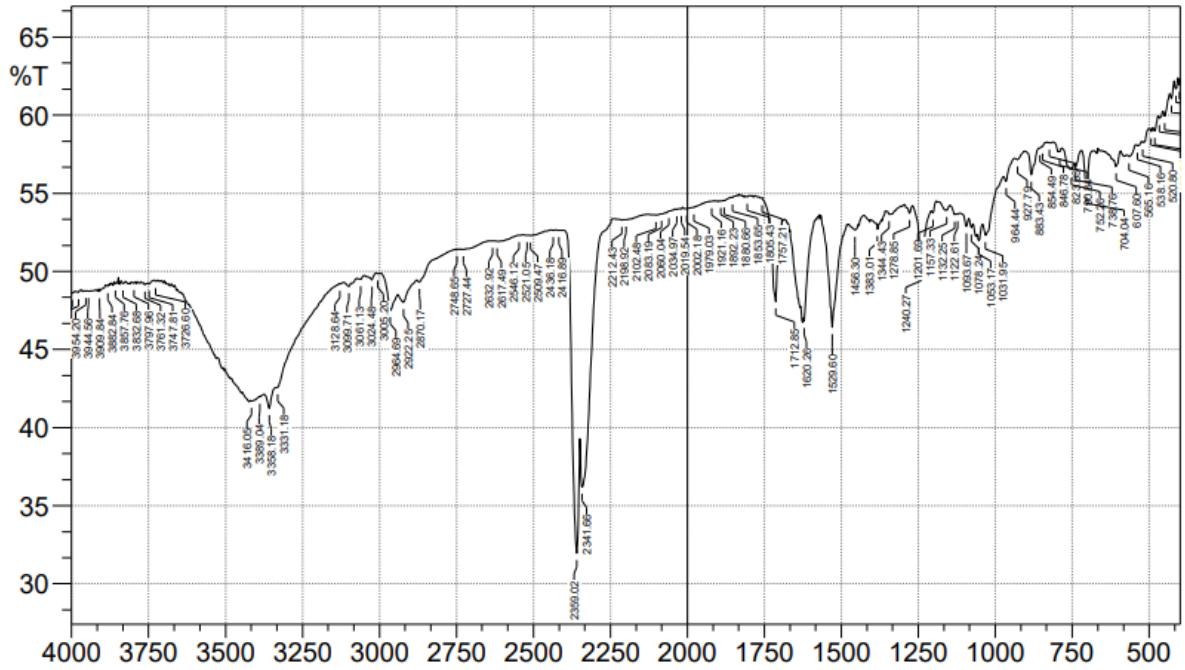
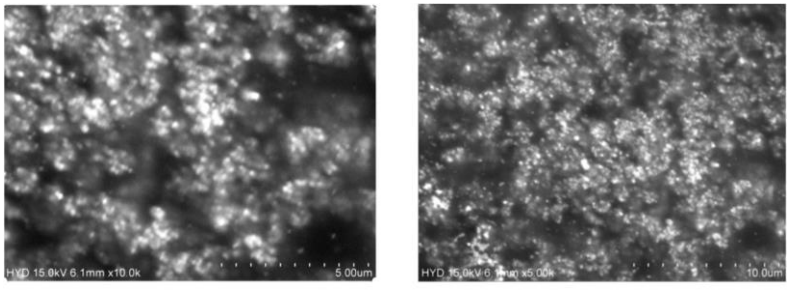


Figure 5: FTIR of optimized formulation (F18)



(6A) **(6B)**

Fig 6: SEM images of optimised formulation of atazanavir optimised SLN F18 (A and B)